PROCEEDINGS
OF THE
NATIONAL ACADEMY OF SCIENCES
Volume 5 APRIL 15, 1919 Number 4

RESTORATION OF VITALITY THROUGH CONJUGATION

GARY N. CALKINS
DEPARTMENT OF ZOOLOGY, COLUMBIA UNIVERSITY

Communicated by Edmund B. Wilson, January 15, 1919

The physiological effect of conjugation, or fertilization, has been interpreted, in the main, along two lines of theory. One of these may be indicated by Bütschli's term *Verjüngung*, and by Maupas's corresponding one *rajeunissement*, terms indicating that the primary effect of conjugation is to restore vital activities to an optimum. The other theory, first fully elaborated by Weismann, assumes that the union of germ plasms (amphimixis), brought about by conjugation, is a source of variations.

These theories are not reciprocally exclusive, and it is possible that both are correct, although neither has been conclusively established.

Bütschli interpreted conjugation in the protozoa, as a means whereby waning vitality is restored to full metabolic activity. The problem thus suggested, involves three fundamental questions: (1) Does the protoplasm of a single individual protozoon and its progeny by division, undergo a progressive waning of vital activities leading to 'old age,' degeneration, and, finally, to natural death? (2) Does conjugation actually restore such weakening protoplasm to a condition of full metabolic activity? (3) If conjugation accomplishes this, what is the explanation of the result?

The first of these three questions was answered in the affirmative by the experiments of Maupas and of numerous subsequent investigators. The second has never been answered conclusively, although strong experimental evidence has accumulated in support of the affirmative. The third question, obviously, is dependent on the second and will be disregarded here.

In the present preliminary paper, I submit the results of experiments made during the last year and a half, which offer a positive answer to the second of these three questions, affording proof that conjugation, in the ciliated protozoon Uroleptus mobilis, actually restores waning vitality to full metabolic vigor. These results are based on the records of the progeny of a

single individual cell, which have been maintained under identical conditions and fed daily with the same standardized culture medium.

The usual method of isolating single individuals each day in fresh culture medium, has been used throughout; five "lines" of individuals of the same ancestry forming a 'series.' Being isolated each day, conjugation never occurs in the culture dishes.

After the daily isolations, the residual individuals are either thrown away, or are put together in larger culture dishes containing fresh culture medium. Here they are kept for a period of two weeks without renewal of the culture medium. At first there is an abundance of food and the organisms multiply by fission until three or four thousand are present. Later, the food becomes exhausted, and, as is well known in some other cases, the transition from rich feeding to starvation induces conjugation, provided the organisms are sexually mature. This procedure constitutes a "conjugation test" which is carried out at weekly intervals.

The number of divisions per day in each of the five lines of a series is recorded; also the sum of the daily divisions, giving a total number of generations to date in each line. The daily records of all lines of a series may then be averaged for successive periods of ten days each, such averages giving a convenient and accurate representation of the relative metabolic activity at different periods of the life cycle.

From time to time, individuals that have undergone conjugation in the weekly conjugation tests, may be isolated to form the beginnings of filial series which are maintained in isolation cultures exactly as in the original series. From the methods employed in these tests, it is evident that such conjugations take place between rather closely-related individuals which are of the same age. The protoplasm affected by such conjugation, also, has had the same history and the same daily treatment throughout, as that maintained in the isolation cultures. After conjugation, a filial series is continued in isolation cultures, transferred daily to the same medium as that used for the parent series, and parallel records are kept in the same way. A filial series, therefore, represents the same original protoplasm as that represented by isolation cultures of the parent series. Any difference in the same calendar periods between the records of the parent series, and those of the filial series, must, therefore, be attributed to the conjugation that has taken place between the closely-related cells of the parent series.

An ideal culture medium for Uroleptus, was found to be an infusion made by boiling a small quantity of flour and a small quantity of fine-cut hay in spring water. This infusion should be used only after twenty-four hours exposure to the air. After the first fifty days of experimenting, this culture medium, made fresh each day, has been used without any alterations.

I have found that the process of 'endomixis' or asexual reorganization with restoration of vitality, occurs in Uroleptus mobilis while encysted. Such encysted stages persist for long periods, and I find that the organisms cannot

be recovered from the cysts until they have been dried for some weeks. The process of reorganization, therefore, cannot escape observation, and it has never occurred in the isolation cultures. The uniformity of results in all of the series outlined below, is sufficient evidence that no other method of asexual reorganization, or parthenogenesis, has occurred in any isolation culture.

A single ex-conjugant from a pair of 'wild' conjugating individuals was isolated on Nov. 24, 1917. It formed the parental series, or, as I shall call it, the '*A* series' and I would explicitly state again, that all results described here, have been obtained with lineal descendants, by division, of this one ancestral cell, and without change of the culture medium.* Five lines were established at the third division and the relative vitality, at successive ten-day periods, is shown in column 1 of table 1. The series ran through 313 generations by division and died out after a long period of progressively reduced vitality, on September 18, 1918.

Conjugation tests were made every week and gave constant epidemics of pairing after the first six weeks. A single pair of conjugating individuals was isolated on four different occasions. In each case the pair was watched until the two individuals had separated. One of the ex-conjugants on each occasion, was then isolated as the starting individual of a filial series. The first of these ex-conjugants formed the filial C series and came from a pair which were in the 78th generation of the parent A series, on February 4, 1918. The second, formed the filial D series which came from a pair of the A series conjugating on March 8, 1918, in the 137th generation. The third, formed the filial H series, which came from a pair conjugating on May 17, 1918, in the 237th generation of the parent A series. The fourth formed the filial J series which came from a pair conjugating on August 12, 1918, in the 311th generation of the parent A series. The parent A series died from exhaustion in the 313th generation, hence these four filial series were taken off at different periods of waning vitality of the parent protoplasm. Each was maintained in five lines and treated exactly like the parent isolation series. Their histories, in successive ten-day periods, are shown in columns 2, 3, 4 and 5.

The history of the C series was similar to that of the parent A series. After a long period of progressively reduced vitality it died on December 31, 1918, in the 348th generation. The D series outlived the parent series but did not live as long, dying after 230 days in the 271st generation. The H series is still alive and is now (January 8, 1919) in the 277th generation. The J series was taken from the parent A series when vitality of the latter was very low, each individual of the parent series dividing only 2.2 times in ten days. The effect of conjugation, as shown by the J series was to increase the division rate to 17.2 times in ten days, while, for the same calendar period, the parent series was dividing at the rate of two-tenths of one division in ten days. The J series is still dividing actively in the 236th generation.

* At the present time (March 15) descendants are still living with unimpaired vigor.

TABLE 1

AVERAGE DIVISION RATE PER INDIVIDUAL, IN TEN-DAY PERIODS

10-DAY PERIODS	A SERIES	C SERIES	D SERIES	H SERIES	J SERIES	F SERIES	I SERIES	L SERIES	PARENTAGE
1	9.8								C series from A78th generation
2	6.6								D series from A137th generation
3	4.8								H series from A237th generation
4	5.4								J series from A311th generation
5	7.8								F series from C86th generation
6	21.0								I series from F143d generation
7	18.6								L series from I199th generation
8	20.8	18.6							
9	18.0	18.8							
10	12.6	16.2							
11	13.2	16.8	16.0						
12	14.8	17.2	18.2						
13	14.6	15.6	16.0			13.4			
14	13.2	14.2	17.0			13.8			
15	15.0	16.4	17.6			16.4			
16	13.6	15.6	18.4			19.8			
17	17.0	17.4	19.0			20.2			
18	12.8	18.0	20.0	19.6		19.6			
19	7.4	13.4	15.2	16.8		15.8			
20	11.0	14.4	16.6	17.8		16.8			
21	7.0	12.8	14.4	15.8		14.0			
22	9.0	10.4	13.4	14.8		12.8			
23	7.6	11.4	14.6	19.1		14.2	18.4		
24	11.2	17.6	18.4	21.4		19.8	22.6		
25	4.8	10.6	12.6	16.4		13.8	19.4		
26	2.2	9.0	10.4	18.0		15.2	11.8		
27	0.2	9.8	9.4	15.6	17.2	12.0	15.0		
28	0.6	8.6	3.4	15.6	16.6	11.8	15.8		
29	0.2	7.6	1.4	15.4	18.6	10.8	14.8		
30	0.0	6.0	0.8	17.8	17.4	12.0	17.6		
31	*Dead*	6.8	0.4	17.8	20.4	14.6	18.0		
32		4.8	0.2	11.0	17.2	9.8	11.6		
33		2.4	0.0	15.4	15.8	9.6	16.4		
34		0.6	0.0	12.6	17.2	12.0	17.4		
35		0.0	*Dead*	7.4	14.6	8.0	14.0	16.0	
36		0.4		2.4	12.4	5.8	12.8	13.2	
37		0.4		1.6	14.4	3.8	13.8	16.2	
38		0.0		1.6	20.6	2.8	20.8	23.2	
39		0.0		0.4	17.0	0.0	15.8	18.6	
40		*Dead*		1.2	12.6	*Dead*	13.0	16.0	

The F series was started with an ex-conjugant of the C series in the 86th generation, and died December 21 in the 317th generation, ten days earlier than the parent series. The I series was taken from the F series in the 143d generation and is still living actively in the 305th generation. The L series came from this I series in the 199th generation and is actively dividing in the 118th generation. An N series (not included in the table) has recently been started from the J series in the 188th generation.

While table 1 by itself, shows clearly enough that conjugation restores vitality to an optimum, the results may be shown still more strikingly by a comparison of longer periods of time whereby minor fluctuations are less conspicuous. I find from the records of the conjugation tests of all series, that conjugation does not begin to take place until from fifty to seventy days from the start of a series. I have chosen the period of the first sixty days, therefore, as representing the period of sexual immaturity. The records show, furthermore, that this is also the period of optimum metabolic activity.

Comparing the mean division rate of a filial series in this first sixty day period, with the division rate of the parent series for the same calendar sixty days, shows the extent, in division activity, to which conjugation has restored vitality to the parent protoplasm. Thus, during the first sixty days of the C series, the five lines had a mean daily division rate of 8.6333, or each individual averaged 1.726 divisions per day, or in ten days, 17.2666 divisions. In the same sixty days, the parent A series, starting at the 78th generation, had a mean division rate of 7.8666 daily, each individual averaging 15.7333 divisions in ten days. The difference, 1.53, indicates the average increase, in number of divisions in a ten-day period, of each individual of the C series, over each individual of the parent A series. In this case the filial generation was taken from the parent when vitality of the latter was near its optimum, and a small discrepancy between parent and offspring is to be expected. With increasing age of the parent, and with corresponding reduction in vitality of its protoplasm, one might reasonably expect that conjugation between two such weakened individuals, would result in a filial generation in which the discrepancy between parent and offspring would remain practically the same as above. The results, however, do not support this expectation; on the contrary, the discrepancy increases with age of the parent protoplasm, as shown in table 2.

It is evident, from the foregoing, that conjugation results in the complete restoration of vitality regardless of the age or the weakened condition of the parent protoplasm, although both parental and filial series are fed at the same times on exactly the same culture medium. This is particularly striking in the case of the J series.

Table 2 also shows that all filial series return to a certain optimum of metabolic vigor as a result of conjugation, a vigor represented by from 17.1 to 17.9 divisions per individual in ten days. From this optimum there is a gradual loss of vitality which is common to all series and which finally leads to death from old age. This is clearly shown in table 3.

COMPARISON OF DIVISION RATES OF PARENT AND OFFSPRING DURING FIRST SIXTY DAYS OF LATTER

	C FROM A SERIES		D FROM A SERIES		H FROM A SERIES		J FROM A SERIES		F FROM C SERIES		I FROM F SERIES	
Dates	2/7 — 4/8		3/8 — 5/7		5/22 — 7/21		8/16 — 10/15		3/28 — 5/27		7/7 — 9/5	
Parent's age	78 generations		137 generations		237 generations		311 generations		86 generations		143 generations	
Mean division rate per day, all 5 lines	8.6333	7.8667	8.5833	7.0666	8.6666	6.2666	8.9666	0.125	8.6000	8.1000	8.5833	6.5666
Average division rate per individual per ten days of period.	17.2666	15.7334	17.1666	14.1333	17.3332	12.5332	17.9332	0.2500	17.2000	16.2000	17.1666	13.1332
Increase per individual per ten days of period.	1.5332		3.0333		4.8000		17.6832		1.0000		4.0334	

TABLE 3

DIVISION RATES IN SUCCESSIVE SIXTY-DAY PERIODS, ALL SERIES

SERIES AND ORIGIN	FIRST 60 DAYS			SECOND 60 DAYS			THIRD 60 DAYS			FOURTH 60 DAYS			FIFTH 60 DAYS			SIXTH 60 DAYS
	Mean division rate per day, all 5 lines	Division rate per individual per 10 days of period	Date	Mean division rate per day, all 5 lines	Division rate per individual per 10 days of period	Date	Mean division rate per day, all 5 lines	Division rate per individual per 10 days of period	Date	Mean division rate per day, all 5 lines	Division rate per individual per 10 days of period	Date	Mean division rate per day, all 5 lines	Division rate per individual per 10 days of period	Date	
A "Wild" exconjugant 11/15/'17	*	*	11/28 to 1/27	8.183 ±0.1985	16.366	1/28 to 3/29	7.183 ±0.1645	14.366	3/29 to 5/28	4.450 ±0.1822	8.900	5/28 to 7/27	0.6667 ±0.1300	1.333	7/27 to 9/25	Dead 9/18
C From A78 2/4/'18	8.6333 ±0.2185	17.266	2/ 9 to 4/ 8	7.9167 ±0.1809	15.833	4/ 9 to 6/ 8	6.4166 ±0.1945	12.833	6/ 8 to 8/ 5	3.9834 ±0.1760	7.966	8/ 5 to 10/ 4	0.7166 ±0.0593	1.433	10/ 4 to 12/ 4	Dead 12/31
D From A137 3/8/'18	8.5833 ±0.1349	17.166	3/ 8 to 5/ 7	8.1666 ±0.2475	16.333	5/ 8 to 7/ 7	5.7834 ±0.2374	11.566	7/ 7 to 9/ 5	0.3500 ±0.0737 (40 days	0.700 only)	9/ 5 to 10/15	Dead 10/15			
H From A237 5/17/'18	8.6666 ±0.1892	17.333	5/22 to 7/21	8.5333 ±0.2165	17.066	7/16 to 9/14	7.0000 ±0.2495	14.000	9/15 to 11/14	Not com	pleted.					
J From A311 8/12/'18	8.9666 ±0.2252	17.933	8/16 to 10/15	7.9167 ±0.1956	15.833	10/15 to 12/14	Not com	pleted.								
F From C86 3/25/'18	8.6000 ±0.2320	17.200	3/28 to 5/27	7.7834 ±0.2313	15.566	5/27 to 7/26	6.3333 ±0.1690	12.666	7/26 to 9/24	4.8833 ±0.2100	9.766	9/24 to 11/23	Dead 12/21			
I From F143 7/1/'18	8.5833 ±0.2468	17.166	7/ 7 to 9/ 5	7.9834 ±0.2320	15.966	9/ 5 to 11/ 4	7.5166 ±0.2256	15.0332	11/ 4 to 1/ 3	Not com	pleted.					
L From I199 11/2/'18	8.7000 ±0.2986	17.400	11/ 4 to 1/ 3	Not com	pleted.											

* Culture medium not established until 55th day.

From this table it is evident that all series follow the same general history, and that, at corresponding periods of the life cycle, all have about the same vigor, although the actual dates may range through all twelve months of the year.

The experiments thus show not only that waning vitality leading to old age and natural death is manifested by Uroleptus mobilis, but also, that conjugation between two individuals at any stage of waning vitality, leads to a complete restoration of vitality.

FALKLANDIA

By J. M. Clarke

State Museum, Albany, New York

Communicated, January 29, 1919

Falklandia is a name herewith applied to a continental land which, during the Devonian period in the occidental parts of the Southern Hemisphere, preceded Gondwana-Land and Antarctis. The history of Gondwana-Land is well established (Neumayr, Suess); its supposed earliest outlines have been approximately determined by the study of its land flora (D. White). The conception of Gondwana-Land is that of a great east-west southern continent which escaped the turmoil of world-wide postcarboniferous deformations and which continued its existence as a continental asylum for land and stream life till late in the Mesozoic time (Cretaceous) when incursions of the sea began which led to its breakdown and demolition in the Tertiary. Eastern Brazil into Sao Paulo, southern Argentine and the north half of the Falkland Islands constitute its western fragments; South Africa, the lost Lemuria (from Madagascar to Ceylon), India and Australia indicate its western extent. Those who have been responsible for the determination of this continent and especially Suess, who has discussed it in much detail, have not recorded its existence prior to the Carboniferous. Antarctis likewise, another Southern 'asylum,' defined on the basis of its terrestrial life and never accurately delimited by its proponents as to the date of its origin, gives proof of like beginning of stabilization and perhaps also of length of endurance. The fossil woods discovered in the Beacon sandstone of South Victoria-Land by James Eights ninety years ago, and the fossils brought home in recent years by Andersson, Nordenskiold, Amundsen, Shackelton and the men of Scott, tend to indicate that it was coexistent in time with Gondwana-Land.

Asylums, thought Suess, were to be defined by continuity in the succession of terrestrial life; it must be added, however, that security of such determinations can be given by the character of the life of the sea which washed the shores of such asylums. Gondwana-Land and Antarctis had a parallel existence in time, though a distinct one. Osborn's observations indicate the